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Bacterial lipopolysaccharides, phorbol myristate acetate, and zymosan induce the myristoylation of specific macrophage proteins

(protein acylation/arachidonic acid metabolism/protein kinase C/signal transduction/membrane attachment)

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ABSTRACT **We demonstrate stimulus-dependent incorporation of exogenously added [^{3}H]myristic acid into specific macrophage proteins. In control unstimulated cells an 18-kDa protein is the major acylated species. In cells incubated with bacterial lipopolysaccharide (LPS), or its monoacyl glucosamine phosphate derivative, fatty acid is incorporated into proteins with molecular mass of 68 kDa and a doublet of approximately 42–45 kDa. Phorbol 12-myristate 13-acetate (PMA) or a phagocytic stimulus (zymosan) promotes the acylation of a similar array of proteins. However, PMA and zymosan also promote the myristoylation of unique proteins of [illegible] and 50 kDa. The fatty acid associated with each of the acylated proteins is myristic acid. The myristate is probably linked to the proteins through amide bonds, since it is not released by treatment with hydroxylamine. Palmitate and arachidonate are not incorporated into proteins in the same manner. Temporal analysis revealed that LPS-induced proteins are myristoylated by 30 min, while the 50-kDa protein myristoylated in response to PMA is labeled later. Most myristoylated proteins appear to be associated with the membrane fraction. Macrophages from C3H/HeJ mice, which do not respond to LPS, do not show any LPS-dependent protein acylation. Interestingly, zymosan and PMA induce the myristoylation of the 50-kDa protein in C3H/HeJ macrophages, but not the acylation of the 68-kDa and 42-kDa doublet species. We suggest that myristoylation of specific proteins is an intermediary in the capacity of LPS, PMA, and zymosan to alter macrophage functions such as arachidonic acid metabolism.**

A major mechanism whereby macrophages mediate inflammation is through the secretion of arachidonic acid (20:4) metabolites (1). When murine resident peritoneal macrophages interact with zymosan particles or with phorbol 12-myristate 13-acetate (PMA) they secrete 20:4 metabolites (2, 3). We have recently shown that treatment of cells with bacterial lipopolysaccharide (LPS) increases the maximal amount of 20:4 release induced by zymosan or PMA and eliminates the lag phase of the response seen with zymosan or PMA alone (4). The active moiety of LPS, lipid A, contains a 3-OH-myristic acid moiety that has been shown to be important in LPS-induced responses (5, 6). Since acylation of select proteins has been described in several cell types (7, 8), we considered the possibility that the acylation of macrophage proteins with the 3-OH-myristic acid moiety of LPS is involved in the effect of LPS on 20:4 release by macrophages. Our first approach was to determine whether stimulation of macrophages resulted in the incorporation of exogenous [^{3}H]myristic acid into specific proteins.

Two general protein acylation reactions have been reported. The first involves the palmitoylation of proteins via ester bonds (review, ref. 9) and the other the amide linkage of myristic acid to proteins (10–14). Many of the proteins that have been shown to have the capacity to be myristoylated are important in cellular regulation, including the catalytic subunit of the cAMP-dependent protein kinase (10), calcineurin B (a component of a calmodulin-binding phosphatase) (11), the pp^{56} tyrosine kinase (12), and the pp60^{src} tyrosine kinase (13, 14). Although the function of the myristic acid moiety in these acylated proteins is unknown, it has been shown in the case of the pp60^{src} to promote the association of the tyrosine kinase with membranes and is required for its transformation properties (13, 14).

In this report we demonstrate that specific proteins are acylated with exogenous myristic acid when macrophages are stimulated with LPS, zymosan, or PMA.

MATERIALS AND METHODS

Macrophage Cultures. Primary cultures of peritoneal macrophages were established from resident cells of specific pathogen-free female ICR mice (Trudeau Institute, Saranac Lake, NY) or from C3H/HeJ mice (The Jackson Laboratory) as previously described (15). Peritoneal cells (approximately 9×10^6 per ml) in α modified minimal essential medium (α-MEM; GIBCO) containing 10% fetal calf serum were cultured in 35-mm-diameter plastic culture dishes (1 ml per dish). After 2 hr at 37°C in 95% air/5% CO_2, cultures were washed three times in calcium- and magnesium-free phosphate-buffered saline (PD) to remove nonadherent cells. The cells were then incubated overnight in α-MEM containing 10% fetal calf serum.

Myristoylation of Macrophage Proteins. Macrophages cultured at a density of approximately 3×10^6 cells per 35-mm culture dish were washed four times with PD and incubated for the indicated times in 1 ml of α-MEM containing [9,10-^{3}H(N)]myristic acid (20–40 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) and the specified stimuli. Stimuli included PMA (Sigma), *Escherichia coli* K-12 LPS (List Biologicals, Campbell, CA), monoacyl glucosamine phosphate (MAGP) (Lipidex, Middleton, WI), and zymosan (ICN) and were prepared, stored, and delivered as described previously (4). At the end of the specified incubation time the cells were washed three times with PD, and scraped into PD containing 1% (wt/vol) Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, aprotinin at 0.28 trypsin inhibitor unit/ml, 1 mM diisopropylfluorophosphate, and 15 mM EDTA (Sigma) (lysis buffer). Nuclei were removed by centrifugation for 5 min in an Eppendorf microcentrifuge and the protein content of the postnuclear supernatants was determined according to the method of Lowry *et al.* (16). Samples containing equiv-

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Abbreviations: 20:4, arachidonic acid; PMA, phorbol 12-myristate 13-acetate; LPS, bacterial lipopolysaccharide; MAGP, monoacyl glucosamine phosphate.

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